EXPLORING CICHLID FISH TOOTH REGENERATION

TURNOVER RATES

A Thesis
Presented to
The Academic Faculty

by

Maya Tome

In Partial Fulfillment
of the Requirements for the Degree
Biology in the
School of Biological Sciences

Georgia Institute of Technology
May 2017

COPYRIGHT 2016 BY MAYA TOME

EXPLORING CICHLID FISH TOOTH REGENERATION

TURNOVER RATES

Approved by:

Dr. Todd Streelman, Advisor
School of Biology
Georgia Institute of Technology

Dr. Eric Gaucher
School of Biology
Georgia Institute of Technology

Date Approved: 12/14/2016

ACKNOWLEDGEMENTS

I would like to thank my graduate student advisors Dr. Ryan Bloomquist and Ms. Teresa Fowler for serving as my mentors during my undergraduate career. Without your support, encouragement, and expert direction, I would not have completed successful data collection nor would I have risen to the high expectations set for myself upon entrance to Georgia Tech. I would also like to thank my principle investigator, Dr. Todd Streelman, for continuing to see my potential and for giving me a place in the research environment.

TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		iv
LIST OF FIGURES		vi
SUMMARY		vii
CHAPTER		
1	Introduction	1
2	Literature Review	5
3	Materials and Methods	8
	Cichlids	8
	Pulse-Chase Experimentation	8
	Alizarin and Calcein Bath Step	8
	Confocal Microscopy	9
4	Goals	11
5	Results	12
6	Discussion & Conclusion	16
7	Future Work	20
REFERENCES		21

LIST OF FIGURES

Page

Figure 1: Chart Illustrating Sequence of Events to Carry Out the Entire Experiment 9

Figure 2: Jaw Orientation in Preparation for Confocal Microscopy 10

Figure 3: Alizarin and Calcein Incorporation into the Cichlid Teeth 13

Figure 4: Visualizing tooth turnover rates via pulse-chase experimentation 14

Figure 5: A model of average tooth density and replacement patterning in *C. afra* and *M. zebra* 18

SUMMARY

Polyphyodonts are organisms who can continuously replace their teeth throughout their lives, yet this mechanism is extremely complex. There are many factors that can affect tooth density and turnover rate, including genetic predisposition and environmental stimuli. In this study, we use pulse chase experiments to investigate the tooth turnover rates of polyphyodont cichlid fish species with differing tooth densities and morphologies. The experiments were carried out with 15-20 day old cichlids from the species *Cynotilapia afra* and *Metriaclima zebra*, which are unicuspid and bicuspid respectively. Alizarin-red and Calcein green fluorescent dye were applied in succession with varied timing, and dye incorporation patterns were then analyzed to distinguish recently replaced teeth from older teeth. *C. afra* have a lower density unicuspid teeth and *M. zebra* have a higher density of bicuspid teeth. Preliminary results suggest that the *C. afra* had slower tooth regeneration when compared to the *M. zebra* jaws, as Alizarin dye incorporation was much more prevalent in the unicuspid jaws; however, the *M. zebra* jaws featured many more half red/half green teeth and a larger number of Calcein-only replacement teeth that began to form a distinct, second row. Thus, after compiling the preliminary results, the data suggests that the unicuspid species *C. afra* has a slower tooth turnover rate than the bicuspid species *M. zebra*, but a larger sample size is needed to confirm these assertions.

CHAPTER 1

INTRODUCTION

The East African cichlid fish from Lake Malawi can be used as a model organism for regenerative tissue research because it constantly replaces its teeth throughout life. This mechanism has been lost in many mammals, and understanding this natural regenerative process can lead to breakthroughs in human regenerative dentistry. Tooth development is a conserved process that requires interaction between two types of cells: an outer layer of epithelial tissue that forms the tooth enamel and the underlying mesenchyme that develops into dentin (Xiao et al., 2014). For humans, this process occurs only twice to form the primary teeth and subsequently the permanent adult teeth, but for cichlids it occurs continuously about every 30-100 days (Fraser et al., 2013) throughout life to yield many sets of teeth.

Many humans have problems with their teeth related to age or decay, so identifying the mechanisms that allow cichlids to continuously replace their teeth has great potential to help many people. The field of regenerative dentistry incorporates the study of stem cells, particularly adult mesenchymal and epithelial stem cells, that play a role in the regeneration or replacement of defective tissues (Xiao et al., 2014). These cells first begin as embryonic stem cells, then differentiate into multipotent stem cells (including the adult mesenchymal cells and epithelial stem cells), and can from there interact with each other to form new structures such as cementoblasts, periodontal ligament cells, odontoblasts, and other dental pulp cells (Xiao et al., 2014). In recent

studies, it has been found that tooth regeneration uses mechanisms similar to those established for embryonic development of structures such as feathers, glands, and hair (Fraser et al., 2013). This is particularly interesting since humans lose this capacity for regeneration at a young age and cannot universally exploit this regenerative mechanism (Fraser et al., 2013). However, in addition to identifying and understanding the necessary stem cells, it is also important to explore the mechanisms that regulate the timing of cichlid tooth turnover and their relationship to the patterning of the jaw. We have observed that different species of cichlid replace their teeth at varying rates, but have never experimentally determined these rates of replacement. There is such variation in tooth numbers among vertebrates, that many questions have yet to be answered concerning the evolutionary importance of environment (including feeding mechanisms), and genetics (including tooth number and shape) on tooth density, as well as how it affects tooth turnover rate.

According to Albertson, Streelman, and Kocher, cichlid feeding strategies can be partitioned into three distinct categories, including: biting, sucking, and ram feeding (Albertson et al., 2003). Upon further analysis of these behaviors, one can see that these specialized modes of feeding have led to diversity in jaw and tooth shapes as well as tooth density across different species of cichlids. In this experiment, I will be analyzing the two cichlid species *Cynotilapia afra* and *Metriaclima zebra*; *C. afra* are planktivores and contain only a small number of unicuspid teeth, whereas *M. zebra* are a rock-dwelling species with a greater number of bicuspid teeth (Bloomquist et al., 2015). There is a difference in which these two species consume food, as the unicuspid planktivores

swallow their prey whole and the bicuspid *M. zebra*s physically break down their food with their flexible or shearing teeth (Bloomquist et al., 2015). These rock-dwellers likely implement more wear and tear on their teeth, which could be an environmental factor that affects their tooth density and turnover rate. Previous studies have explored how teeth evolved, and whether tooth number convergently evolved with tooth turnover rate. The results of these studies show that when comparing freshwater populations of Stickleback fish to their ancestral marine populations, there is a significant change in the tooth replacement cycles (Ellis et al., 2015). They also found that there was a significant difference in tooth number between the two species' ventral pharyngeal jaws (Ellis et al., 2015), which develop through similar genetic mechanisms to those of the oral jaws (Fraser et al., 2009). Overall, the freshwater populations seemed to replace their teeth much quicker than their ancestral marine counterparts, so this ties in to how both genetic and environmental factors affect tooth turnover rates.

In this study, I analyzed and compared *C. afra*, which have a low density of unicuspid teeth, to *M. zebra* cichlids, which have a higher density of bicuspid teeth. I hypothesized that I would find the rate of tooth replacement greatly depends on shape and density; but more specifically, that *M. zebra* replace their teeth more frequently than *C. afra*, similar to the association between increased tooth number and increased rates of new tooth formation found in Stickleback species (Ellis et al., 2005). In addition, the genetic model of morphological integration is based on the observation that traits will be inherited together based on their function (Albertson et al., 2005); so, when I compared the cichlids with a low density of unicuspid teeth to cichlids with a higher density of

bicuspid teeth, I expected to see the genetic model of morphological integration at work and that the cichlids' feeding patterns would greatly affect their tooth density and furthermore their tooth turnover rates.

CHAPTER 2

LITERATURE REVIEW

Regenerative dentistry, which centers around the development of stem cells, is a rapidly growing field since stem cells are critical in organ and tissue growth and repair (Xiao et al., 2014). Stem cells begin as totipotent, and once they differentiate into multipotent cells, they can then begin to build vital structures including tissues and organs. Adult epithelial and mesenchymal stem cells work together to instigate tooth development, which is unique from processes like organ growth as this type of replacement is non-life-threatening (Xiao et al., 2014). This key feature is conducive to furthering research in the field of regenerative dentistry, and it has helped discover features of these stem cells—like how they ultimately differentiate into numerous structures such as teeth, taste buds, and salivary glands in very defined locations (Bloomquist et al., 2015). Teeth and tooth nerve evolution is also currently being studied, and according to Ellis et al., tooth replacement in polyphyodont vertebrates is believed to be controlled by the same genetic network that governs primary tooth formation (Ellis et al., 2015). This mode of tooth renewal is demonstrated in cichlids, who are also polyphyodonts, and this pattern can be studied in both the oral and the pharyngeal jaws. Many different fish possess these two sets of tooth-covered jaws, with the oral jaw serving as a mechanism to primarily capture prey and the pharyngeal jaw aiding in digestion (Ellis et al., 2015). Overall, both genetic and environmental factors affect tooth turnover rates, but extensive research still has yet to be done to answer many questions in this field.

Although many studies have been done in regards to confirming what molecules and pathways are involved in individual organ identification, little research has been done on tooth patterning (Bloomquist et al., 2015). More specifically, we have noted that tooth replacement rates vary among cichlid species, but have neither determined these rates nor compared them to each other. In addition, many different hypotheses have been formulated to try and understand the mechanism and the workings behind tooth regeneration, including one by Albertson, Streelman, and Kocher. They argue that there is strong directional selection influencing the cichlids' oral jaws and teeth (Albertson et al., 2003), and they also continue to prove that there is a divergence between species of bony fish that feed either on stationary or mobile prey, and that this has led to a clear deviation within the evolution of the stereotypical feeding apparatus (Albertson et al., 2005). Cichlids display three distinct modes of feeding, and these are believed to have led to the diversification in jaw and tooth shapes. After looking at the research that has been conducted today, it is clear that tooth density and turnover rate has remained a major un-answered question—and that is what this experiment hopes to resolve.

To answer this question, I will perform pulse-chase experiments with Alizarin Red and Calcein fluorescent dyes. From there, I want to compare the tooth densities and turnover rates between species with confocal microscopy. By using confocal microscopy, I will be able to take 3-D images of both the oral and pharyngeal jaws without having to interfere with the integrity of their structures (Rajadhyaksha et al., 1995). Once I am able to gather enough high-quality photos via confocal microscopy of jaws pulsed with varied

timing, I will compare dye incorporation to determine tooth turnover rates. Ideally, I will collect confocal images of the cichlid teeth at different points of dye incorporation, plot and look for trends, and diagram areas of tooth growth for *Cynotilapia afra* and *Metriaclima zebra*, which are unicuspid and bicuspid species respectively.

CHAPTER 3

MATERIALS AND METHODS

Cichlids

This study focused on polyphyodonts, organisms who replace their teeth continuously throughout life. We chose to study the East African cichlid fish from Lake Malawi, and more specifically, our experiments incorporated 15-20 day old cichlids from the species *Cynotilapia afra* and *Metriaclima zebra*. These species are are unicuspid and bicuspid respectively, which refers to the shape of their teeth.

Pulse-Chase Experimentation

To test my hypothesis, I performed pulse-chase experiments by applying Alizarin Red and Calcein fluorescent dyes, which bind to calcium in the teeth. By differing the application time of these dyes and the length of chase, I manipulated their fluorescence signals to show tooth turnover rates. This is because pulse-chase experiments were created to exploit the dynamics of cell regeneration; since cells were designed to replicate and degrade at different times, pulsing fluorescent dyes and allowing for chase time enabled cell localization and expression levels to be tagged over time (Gautier et al., 2008).

Alizarin and Calcein Bath Step

To begin the pulse-chase experiment, 8 mL of a 0.01% Alizarin-red dilution was combined with 150 mL of water (also referred to as fish water) to make an Alizarin wash for the cichlids. We placed 15-20 day old cichlids in the bath in the dark and allowed

them to remain in the Alizarin for 24 hours, subsequently replacing it with regular fish water. The first phase involving the Alizarin wash is known as the pulse step, and this was then followed by a two-week period in which the cichlids remained in the fish water and were given time for their teeth to grow. After this 14 to 16-day period, 25 mL of a 0.005% Calcein green dilution was combined with 100 mL of fish water to make a Calcein wash for the cichlids. We placed them in the bath in the dark and again allowed them to remain in the Calcein for 24 hours, subsequently replacing it with regular fish water. This was then followed by a one-week period in which we placed the cichlids in fish water once again to allow time for tooth growth (Figure 1).

Figure 1: Chart illustrating sequence of events to carry out the entire experiment

Confocal Microscopy

From there, my goal was to compare the turnover rates between species with different tooth densities via confocal microscopy to determine the validity of my hypothesis. Confocal microscopy is a method by which I was able to analyze cichlid jaws and take images of their 3-D structures by focusing on cross-sections without compromising the integrity of the tissue samples (Rajadhyaksha et al., 1995). After each pulse-chase experiment was complete for a collection of cichlids, each cichlid was individually dissected so that the top, bottom, and pharyngeal jaws could be analyzed. I used a sharp scalpel and a pair of tweezers to carefully cut out each cichlid's jaws, and they were subsequently mounted onto a concave microscope slide. Each well was filled with Vectashield to prevent photo bleaching, so once the Vectashield and the jaws were placed in a well, each jaw had to be properly oriented. In order to be successfully

examined under the microscope, a jaw had to sit on its side in the well so that the entire structures of the teeth could be seen (Figure 2). From there, each well was sealed with a coverslip, and the slides were then analyzed via confocal microscopy.

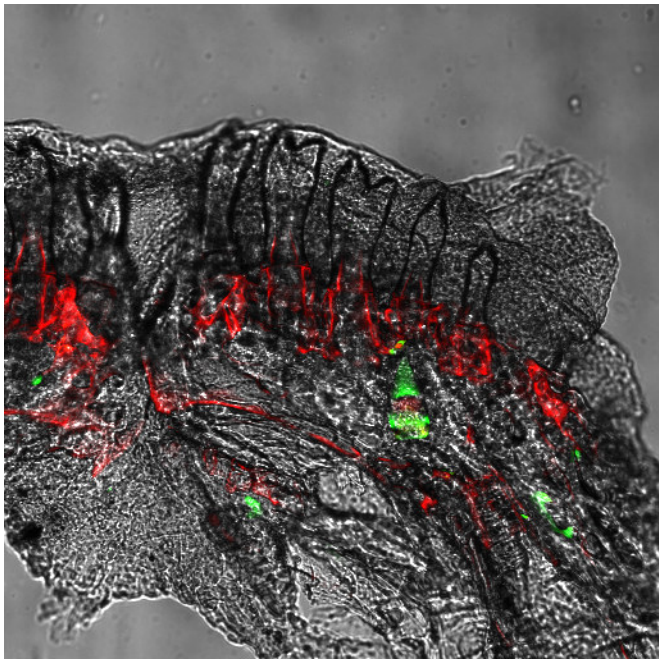


Figure 2: Jaw orientation in preparation for Confocal Microscopy. An *M. zebra* jaw was mounted onto the concave slide on its side. This allowed the full structures of each of the teeth to be visible via Confocal Microscopy.

CHAPTER 4

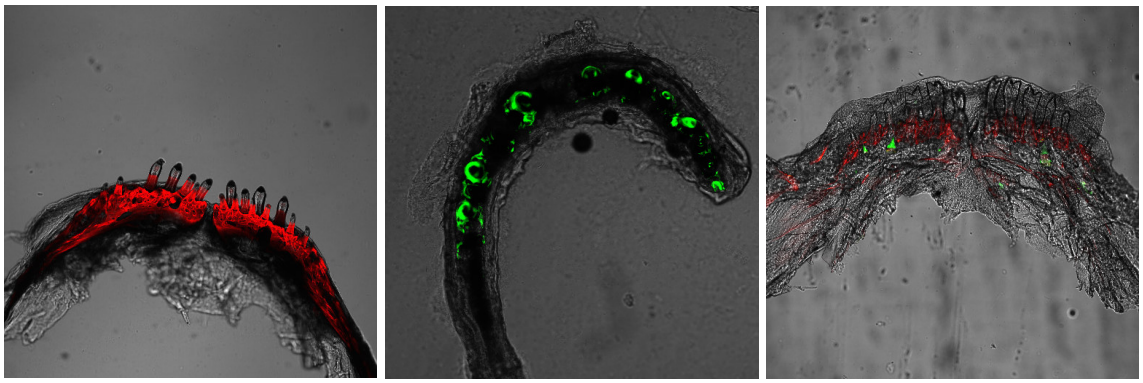
GOALS

I hypothesized that I would find the rate of tooth replacement greatly depends on shape and density, and that *M. zebra* replace their teeth more frequently than *C. afra*. To test this hypothesis, I performed pulse-chase experiments with Alizarin Red and Calcein fluorescent dyes. Immediately after the first bath with Alizarin dye, all of the teeth should glow red. Over the chase period, these functional teeth will grow so that the tips remain red but the base of the teeth, which were below the jaw tissue during dye application, will not have incorporated the dye. During the chase, some of these red functional teeth will be shed and replaced by new teeth, which will not have incorporated the red dye. This is because cichlid fishes replace the teeth in their oral jaws every 30-100 days (Fraser et al., 2013), so I will be using cichlids that are approximately 15-20 days old in order for the teeth to grow and begin replacement without completing a full turnover cycle. In this way, I will be able to identify the newly replaced teeth and determine if there are certain areas of the jaw that replace faster based on the number of red teeth versus the number of teeth without red. The same principle will work for the green dye, so that once both have been incorporated and chased, the teeth that were present since the initial pulse will have red tips and green bases, while new teeth will be completely green. Thus, in cichlids who have a higher density of bicuspid teeth, I expect to see more Calcein-incorporated, green teeth because they will have replaced their original teeth that had incorporated the Alizarin Red dye quickly. On the other hand, in cichlids who have a lower density of unicuspid teeth, I expect to see many more teeth that are half red, half green, indicating that they replace their teeth more slowly and have not yet replaced their original teeth since the experiment began.

CHAPTER 5

RESULTS

Initially, my goal was to figure out a way to successfully incorporate the Alizarin and Calcein dyes into the cichlid teeth. This involved experimenting with dye and bath concentrations, since a diluted bath resulted in insufficient incorporation, but an overly concentrated bath put the cichlids at risk of death. Once I determined the optimal concentrations of 100 μg/mL and 50 μg/mL for Alizarin and Calcein respectively, I set out to determine the best timing of the pulse-chase experiment, as well as a successful method to mount the jaws in the correct plane on the coverslip. After exploring different timing techniques, I found that the best method included a 24-hour Alizarin bath for the pulse step, 14-16 days in fresh fish water, another 24-hour bath with Calcein, and another 3-4 days in fresh fish water. This alternation in the timing and the application of the dyes lead to a clear visualization of both the Alizarin and the Calcein dyes in the cichlid teeth (Fig. 3). In addition, I was able to mount the jaws once the cichlids were done with the pulse-chase experiment in a reproducible plane that yielded images conducive to analysis.



A **B** **C**

Figure 3: Alizarin and Calcein incorporation into the cichlid teeth. (**A**) *C. afra* jaw that had a 2-week Alizarin dye incorporation period. (**B**) *M. zebra* that had a 1-week Calcein dye incorporation period. (**C**) *M. zebra* jaw in which older teeth display the Alizarin dye and the younger, replacement teeth display the Calcein dye.

Once optimal timing and concentration rates were found, complete pulse-chase experiments (including both Alizarin and Calcein pulses) could be performed on both *C. afra* and *M. zebra* species. Photos were then taken to visualize red-green patterning, confirm tooth density and spatial placement, and ultimately use this information during analysis to determine tooth turnover rates or pattern differences between the uni- and bicuspid species. As shown in Fig. 4, photos of both species were collected and Alizarin and Calcein incorporation could be seen as well as teeth that were half red/half green colored.

M. zebra

C. afra

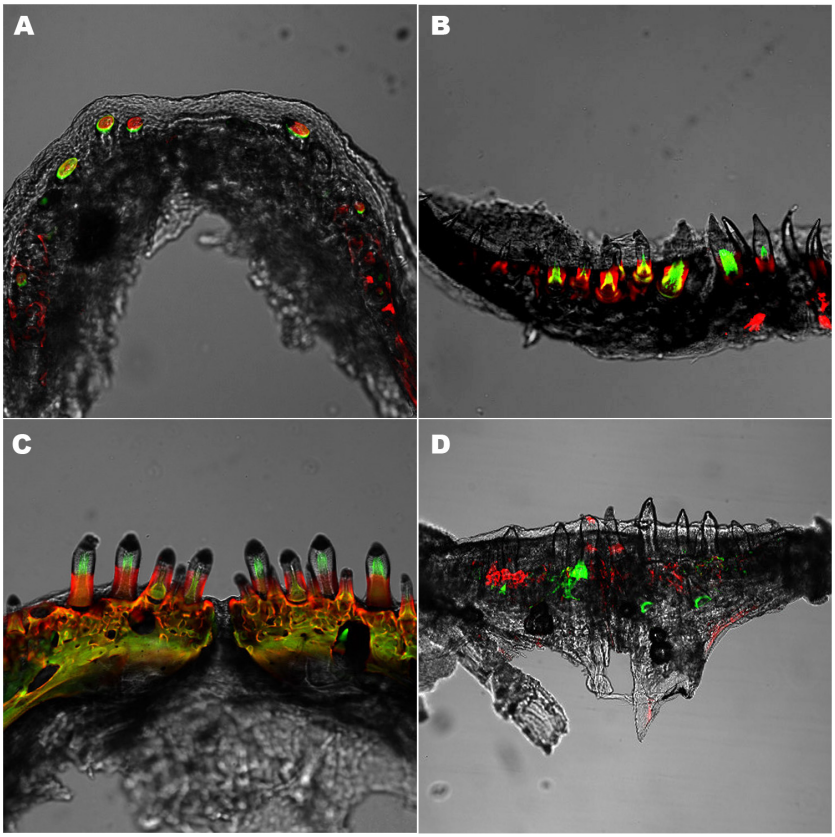


Figure 4: Visualizing tooth turnover rates via pulse-chase experimentation. (**A**) *M. zebra* jaw in which the pulse-chase procedure was performed over 3 weeks. Alizarin dye can be seen in the tips of the teeth and Calcein appears closer to the base of each tooth. (**B**) *M. zebra* jaw that shows how this species typically had both Alizarin and Calcein incorporation. Many of the teeth were half red/half green. (**C**) *C. afra* jaw in which the experiment was performed over 2.5 weeks. Alizarin dye can be seen at the base and around the teeth, while Calcein appears in the core of each tooth. (**D**) *C. afra* jaw that shows how this species typically had more Alizarin-only and Calcein-only incorporation in the teeth. Alizarin can be seen in the larger teeth and Calcein appears in the core and at the base of the smaller, replacement teeth.

In order to determine turnover rates and patterns of replacement, many photos had to be taken via confocal microscopy and then subsequently analyzed. After comparing nine intact *C. afra* jaws of 25-30-day old fish, I observed an average tooth density at this age of 11 teeth. The majority of these teeth comprised the front row of the jaw, and

typically only one or two teeth were seen to be forming in a second row. In comparison, I also analyzed nine intact *M. zebra* jaws of 25-30-day old fish and found that the average tooth density at this age was 17 teeth. The majority of these teeth were also placed in the front row of the jaw, and three to four teeth were seen to be forming a second row. Overall, although the sample size was small, *M. zebra* jaws had greater Calcein incorporation to form half red/half green teeth as well as Calcein-only green teeth than the *C. afra* jaws.

CHAPTER 6

DISCUSSION & CONCLUSION

Once the average densities for *C. afra* and *M. zebra* were found (Fig. 5), the photos were next analyzed for Alizarin-Calcein incorporation patterning. Finding these patterns required undamaged jaws with full Alizarin and Calcein incorporation, as well as correct mounting for clear visualization under the confocal microscope. However, the small sample size did yield suggestive results that are promising.

After analyzing three *C. afra* jaws, several patterns were observed. First, the teeth were largest in the middle of the jaw, and they decreased in size as the teeth formed further back in the jaw. The teeth in the front row were larger than those that appeared to form a second row, suggesting that the front row forms first beginning in the middle of the jaw and working its way back. Next, analysis of the Alizarin and Calcein incorporation was executed by recording which dye was present in each tooth position. This analysis showed that on average, the teeth in the middle of the front row tended to be red, with half red/half green teeth towards the middle and back of the jaw, and with green-only teeth appearing in the very back of the jaw and in the beginnings of a second row. This again suggests that the front row forms before the second row, and that teeth begin to form in the middle of the jaw and work their way back. Also, the *C. afra* teeth tended to show exclusively Alizarin incorporation more often than Calcein. This indicates that the tooth turnover rate was slow and that the teeth did not replace quickly enough to show Calcein-only replacement teeth (Fig. 5).

Several patterns were similarly observed after analyzing three *M. zebra* jaws. First, like *C. afra*, the front row seemed to form before the second row since the majority of teeth appeared in the front row, and only smaller, replacement teeth formed in a second row. The teeth in the front row were larger towards the middle and decreased in size as they appeared further back in the jaw. Going along with this pattern, since *M.*

zebra cichlids are bicuspid, it became evident that they don't develop their normal bicuspid shape until after a few rounds of replacement have passed. The teeth in the front row towards the middle of the jaw displayed the bicuspid shape most often, and then the teeth appeared to be unicuspid more often the further back they were positioned in the jaw—this suggests that the front teeth have been replaced more than the back teeth, since they have begun to show the bicuspid shape. A similar observation is supported by Huysseune and Sire, who found that in many cichlids, first generation teeth tended to be smaller and unicuspid, so it was not until further growth and replacement did certain species begin to develop their characteristic tooth shape (Huysseune and Sire, 1997). It wasn't until these teeth had gone through multiple rounds of replacement that they developed the complex shape, innervation, and patterning set during initiation (Streelman et al., 2003 and Fraser et al., 2008). Next, Alizarin and Calcein incorporation was analyzed from the three *M. zebra* jaws. In comparison to the *C. afra* jaws, *M. zebra* on average displayed many more half red/half green teeth. There did not seem to be a pattern of which teeth displayed the Alizarin or Calcein more often, but Calcein-only teeth tended to be towards the back of the jaw and in the second row (Fig. 5).

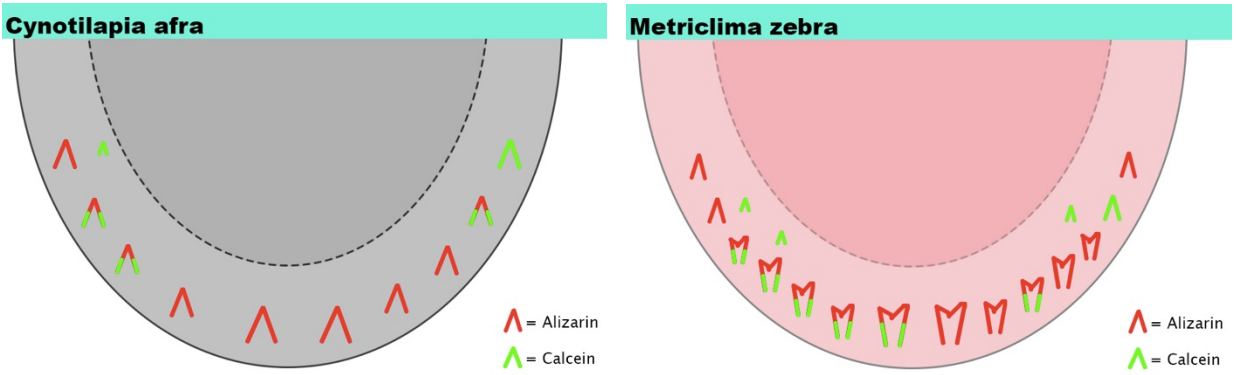


Figure 5: A model of average tooth density and replacement patterning in *C. afra* and *M. zebra.* On the left, the schematic shows that on average, *C. afra* had a low density of unicuspid teeth, with larger sizes towards the middle and decreasing in size as they appear further back on the jaw. Half red/half green teeth were generally observed towards the back of the jaw, and the front row tended to replace more slowly, as Calcein-only replacement teeth appeared more frequently in a second row. On the right, the schematic shows that on average, *M. zebra* displayed a higher density of bicuspid teeth, again with larger sizes towards the middle and decreasing in size as they appear further back on the jaw. The bicuspid shape was only observed in the front row, in teeth that were larger and appeared to be older based on their retention of Alizarin dye. Calcein-only replacement teeth generally developed in the second row towards the back of the jaw, and they remained strictly unicuspid in shape. In addition, half red/half green teeth were observed along the entire front row, without a clear spatial pattern.

These observations indicate that overall, *M. zebra* has a faster tooth turnover rate, primarily because Calcein was incorporated into the teeth more often. As seen in Figure 5, a schematic was created to show the average tooth density, positioning, shape, and replacement patterning via Alizarin and Calcein incorporation. When comparing the two species side by side, it is evident that *M. zebra* jaws had greater Calcein incorporation to form half red/half green teeth as well as Calcein-only green teeth. This suggests that *M. zebra* teeth grew at a quicker pace and ultimately formed new replacement teeth more often than *C. afra*, which aligns with my original hypothesis that *M. zebra* cichlids with a

higher density of bicuspid teeth have a higher tooth turnover rate than *C. afra* cichlids, who have a low density of unicuspid teeth.

CHAPTER 7

FUTURE WORK

With these results, many more tests need to be done in order to increase sample size and determine significant results; from there, future studies will include additional cichlid species. *C. afra* will be compared to *Labidochromis caeruleus*, another unicuspid species to see if there are similar tooth turnover rates between the two species. In addition, the uni- and bicuspid turnover rates from this study would be compared to that of *Petrotilapia chitimba*, a tricuspid species. We hypothesize that this species will replace their teeth the most often. Finally, tooth growth and replacement would be analyzed in the pharyngeal jaw, which is located in the cichlid's throat. These turnover rates are projected to be similar to those of the mandible jaws, but no analysis was done on the pharyngeal jaws in this study.

REFERENCES

Albertson, R.C., Streelman, J.T., and Kocher, T.D. (2003). Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. Proceedings of the National Academy of Sciences of the United States of America *100*, 5252-5257.

Albertson, R.C., Streelman, J.T., Kocher, T.D., and Yelick, P.C. (2005). Integration and evolution of the cichlid mandible: The molecular basis of alternate feeding strategies. Proceedings of the National Academy of Sciences of the United States of America *102*, 16287-16292.

Bloomquist, R.F., Parnell, N.F., Phillips, K.A., Fowler, T.E., Yu, T.Y., Sharpe, P.T., Streelman, J.T. (2015). Coevolutionary patterning of teeth and taste buds. Proceedings of the National Academy of Sciences of the United States of America *112*, 5954-5962.

Ellis, N.A., Glazer, A.M., Donde, N.N., Cleves, P.A., Agoglia, R.M., and Miller, C.T. (2015). Distinct developmental genetic mechanisms underlie convergently evolved tooth gain in sticklebacks. Development *142*, 2442-+.

Fraser, G. J., Bloomquist, R. F., and Streelman, J. T. (2008). A periodic pattern generator for dental diversity. *BMC Biology*, *6*, 32.

Fraser, G. J., Hulsey, C. D., Bloomquist, R. F., Uyesugi, K., Manley, N. R. and Streelman, J. T. (2009). An ancient gene network is co-opted for teeth on old and new jaws. PLoS Biol. 7, e1000031.

Fraser, G. J., Bloomquist, R. F. and Streelman, J. T. (2013). Common developmental pathways link tooth shape to regeneration. Dev. Biol. 377, 399– 414.

Gautier, A., Juillerat, A., Heinis, C., Correa, I.R., Jr., Kindermann, M., Beaufils, F., and Johnsson, K. (2008). An engineered protein tag for multiprotein labeling in living cells. Chemistry & Biology *15*, 128-136.

Huysseune, A., Sire, J.Y. (1997). Structure and development of first-generation teeth in the cichlid Hemichromis bimaculatus (Teleostei, Cichlidae). Tissue & Cell *29,* 679-697.

Rajadhyaksha, M., Grossman, M., Esterowitz, D., and Webb, R.H. (1995). In-Vivo Confocal Scanning Laser Microscopy of Human Skin - Melanin Provides Strong Contrast. Journal of Investigative Dermatology *104*, 946-952.

Streelman, J.T., Webb, J.F., Albertson, R.C., Kocher, T.D. (2003). The cusp of evolution and development: a model of cichlid tooth shape diversity. Evolution and Development *5:* 600-608.

Xiao, L., & Nasu, M. (2014). From regenerative dentistry to regenerative medicine: progress, challenges, and potential applications of oral stem cells. *Stem Cells and Cloning : Advances and Applications*, 7, 89-99.